ANNUAL INTERIM REPORT

PROJECT NUMBER: LK0908 **REPORTING YEAR: 2001** *01/01/01 – 31/12/01*
TITLE OF PROJECT: Novel Strategies for aphid control using entomopathogenic fungi

LEAD PARTNER: IACR-Rothamsted

SCIENTIFIC PARTNERS: University of Newcastle

START DATE AND DURATION: January 1st 2000, 4 years

PROJECT OVERALL AIM:

To identify the factors in farmland which affect the abundance and movement of entomopathogenic fungi and manipulate these factors to increase fungal infection in summer aphid populations in cereals, brassicas, legumes and potatoes.

OBJECTIVES ADDRESSED DURING THE REPORTING YEAR:

1.2 To monitor the spatial and temporal population dynamics of aphids and *E. neoaphidis* throughout the year in existing field margins. Isolate the fungus from the field *in vitro*.
2.2 Determine the relative susceptibility of three crop aphid species to *E. neoaphidis* on different host plant species.
3.2 Quantify molecular differences between *E. neoaphidis* isolates collected from adjacent and distant locales in the UK. Measure biological traits.

RESULTS FROM THE REPORTING YEAR:

Results from the reporting year (Jan-Dec 2001):
All milestones have been achieved or exceeded.

1.2 ***Spatial and temporal dynamics of aphids and E. neoaphidis*:** Intensive studies were undertaken in Stubbings field, IACR-Rothamsted farm. Aphid numbers in the field margin and the adjacent wheat crop were low and the prevalence of fungal infection was also very low. *Erynia neoaphidis* was detected in *Sitobion avenae*, along with another aphid pathogenic fungus, *Entomophthora planchoniana.* Studies of infections in nettle aphid (*Microlophium carnosum*) were carried out under the oak tree by the field entrance, continued from last year, as well as a new study initiated to look at nettle aphid populations around the perimeter of Stubbings field. The latter study will support the link between future genetic analyses and field sampling to quantify the spread of *E. neoaphidis* within and between nettle aphids, field margin aphids and crop aphids. Field margins were also monitored at UAP farm, Oxfordshire and Unilever farm, Bedfordshire. Numbers of aphids were low in both crops and margins at both farms. Field work in both 2000 and 2001 indicated that the following plants and aphids had good potential as reservoirs of *E. neoaphidis*: nettles with nettle aphid, *M. carnosum*, teasel with rose aphid, *Macrosiphum rosae*, creeping thistle with black bean aphid, *Aphis fabae* species complex. In all field studies parasitoid wasps have also been recorded for inclusion in future analyses. ***Collection of Erynia neoaphidis isolates:*** Two new isolates of *E. neoaphidis* were collected, preserved and incorporated into the aphid host range bioassays completed last year (see below). The isolates originated from pea aphid, *Acyrthosiphon pisum*, at Rothamsted and although they were collected from the same field were different in their relative pathogenicity to the aphids tested.

2.2 ***Host-plant influence*:** Effects of host plant were tested by comparing infection of aphids on host plants which were used for continuous, routine, culturing of aphids and alternative host plants on

which aphids had been reared for about one month.The aphid species examined were *A. pisum* (original plant = broad bean, alternative = pea), *M. dirhodum* (original = barley, alternative = wheat) and *M. persicae* (original = Chinese cabbage, alternative = potato). Statistical analyses showed that infection was greatest for aphids reared on the original host plant and least on the alternative plant. In addition, pathogenicity assays from the previous year, against seven crop aphid species, were extended to include dose-response studies for three isolates against *A. pisum, M. dirhodum* and *M. persicae*. Values for LD50 obtained from these studies were then tested against the remaining four aphid species and data has yet to be analysed. This approach allows for statistical analysis and provides a robust methodology for testing the susceptibility of other aphid species in the future.

Quantify molecular differences between E. neoaphidis isolates collected from adjacent and distant locales in the UK. DNA was extracted and compared from a further ten isolates, predominantly from cereal aphids, which confirmed last years results that the ITS regions of all *E. neoaphidis* isolates were similar. The full sequence of the ITS region for three isolates of *E. neoaphidis* was determined and found to be very highly conserved. It was therefore impossible to develop isolate specific primers based on small variations in the ITS region between isolates. However, using ERIC, RAMS and RAPD primers differences in banding pattern between groups of isolates of *E. neoaphidis*, including UK isolates, could be observed. A preliminary cluster analysis based on the ERIC results demonstrated the relatedness of isolates; most UK isolates segregated together. ***Measure biological traits.*** The growth of 15 isolates (nine from UK, three from Europe) of *E. neoaphidis* at eight constant temperatures (4 ^{O}C - 30 ^{O}C) was compared. Although full analysis is not complete most isolates had temperature optima between 18 and 22 ^{O}C but varied in their rate of growth. Many isolates were able to grow at 4 ^{O}C, some at similar rates to their growth at 18 ^{O}C. No isolates grew at 30 ^{O}C. The intra- and extra-cellular enzyme production of six isolates was compared. In general, isolates varied in the types and quantities of enzymes that they were able to produce and further studies are underway to relate this to other biological attributes, particularly virulence. The impact of a range of currently used fungicides against germination, growth and sporulation of *E. neoaphidis* was determined. Overall, isolates varied in their sensitivity to the chemicals and the effects were complex, depending on the fungicide and which part of the fungal lifecycle was considered.

CONCLUSIONS AND IMPLICATIONS FOR LEVY PAYERS:

Both existing and newly established field margins have been monitored throughout the season and, although it was again a poor year for aphids, two new isolations of *E. neoaphidis* were collected. Laboratory bioassays have demonstrated variability in susceptibility to infection between the aphid species examined which will have implications for spread of the fungus in the field. The most susceptible species were *M. dirhodum* (cereals), *A. pisum* (legumes) and *M. persicae* (brassicas). The host plant that aphids are reared on was found to affect their susceptibility to infection by *E. neoaphidis*. Other biological attributes of a range of *E. neoaphidis* isolates have also been quantified. These include growth at different temperatures, enzyme production and sensitivity to fungicides.

Primer combinations based on the ITS region of the DNA were useful for distinguishing between different species of fungi but did not separate isolates within the same species. They can therefore be used for determining whether *E. neoaphidis*, as a species, is present or not in aphids collected in the field but not which isolate is present. Additional primers tested have been able to distinguish between groups of isolates of *E. neoaphidis* and have the potential to be used to track specific isolates in the field.

Field sampling regimes, laboratory bioassays and molecular characterisation techniques are being used to provide spatial and temporal data on the occurrence and abundance of *E. neoaphidis* in field margins and crops, the host range of specific isolates and also allow those isolates to be tracked in the field. This will underpin the development of effective field margin management strategies, which will enhance the impact of *E. neoaphidis* on pest aphids in adjacent crops (cereals, brassicas, legumes and potatoes). This will provide additional 'pest management' benefit to existing Countryside Stewardship schemes and reduce the number of applications of insecticide necessary in field crops with the associated economic and environmental benefits.